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Complexation of Pinosylvin, an Analogue of Resveratrol with High Antifungal and Antimicrobial Activity, by Different Types of Cyclodextrins

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The complexation of pinosylvin, a potent antimicrobial and antifungal stilbenoid, by cyclodextrins (CDs) is described for first time in this work. Steady-state fluorescence was used to demonstrate that natural (α-, β-, and γ-CD) and modified (HP-β-CD, methyl-β-CD, and ethyl-β-CD) CDs are able to complex pinosylvin following a 1:1 stoichiometry. However, substantial differences in the strength of the complexation exist between the CDs tested. Although among natural CDs the interaction of pinosylvin with β-CD was more efficient than with α- and γ-CD, the results show that the complexation constants (K_F) were higher for all of the modified CDs than for natural CDs, the highest K_F being that determined for HP-β-CD−pinosylvin complexes (12112 ± 761 M^{-1}). Moreover, deprotonation of the hydroxyl group of pinosylvin led to a sharp fall in the K_F values with respect to those observed for the complexes formed between the protonated structure of this stilbenoid and the CDs. Moreover, a pK_a value is reported for the first time for pinosylvin. Furthermore, when the temperature of the system was increased, a significant drop was observed in the complexation constant values. From these K_F values and to throw light on the mechanism of pinosylvin affinity for HP-β-CD, three thermodynamic parameters, $\Delta H°$, $\Delta S°$, and $\Delta G°$, were calculated. The results show that the complexation of pinosylvin by HP-β-CD is a spontaneous and exothermic process with negative values for entropy changes. Finally, to gain information on the effect of the structure of different compounds belonging to the stilbenoid family on the K_F values, the complexation of other molecules such as (*E*)-resveratrol and pterostilbene was studied and compared with the results obtained for the HP-β-CD−pinosylvin complexes.

KEYWORDS: Pinosylvin; cyclodextrin; fluorescence; resveratrol; pterostilbene

INTRODUCTION

Cyclodextrins (CDs) are nonreducing cyclic glucose oligosaccharides. There are three natural CDs, α-, β-, and γ-CD, with six, seven, or eight D-glucopyranonsyl residues, respectively, linked by α-1,4-glycosidic bonds (*1*, *2*). The double characteristics of CDs, (a) the existence of a hydrophobic cavity and (b) the presence of two hydrophilic hydroxyl rims, give them the property to form inclusion complexes in water with a variety of organic molecules. Because CDs are able to increase the bioavailability of different compounds and to protect different molecules against the action of external agents, their use in both the pharmaceutical and food industries is increasing (*3*, *4*).

Among these applications for CDs, recent years have seen an increased number of papers and patents concerning their use in packaging. Thus, the application of CDs in smart and active food packaging has been reviewed (*5*). Over the past decade, active, controlled, and intelligent packaging techniques have seen significant growth and change as new products have replaced the traditional forms of food and beverage packaging. By mixing CD complexes of fragrances, dyes, insecticides, UV filters, etc. into molten thermoplastic polymers, improved packaging material (films, laminates, containers, trays, etc.) can be produced, in which the complexed substances are homogeneously dispersed and only slowly released from the polymer matrix (*6*). The incorporation of CDs or CD complexes into a plastic packaging material makes it, at least partially, biodegradable (*7*). The advantages of CD application in plastic packaging can be inclusion of a byproduct of polyethylene generated by a heat seal (*8*), the decreased release of impurities and undesired volatile byproducts formed during manufacture of the packaging material into the food or beverages (*9*), improvements in barrier functions of the packaging material entrapping both penetrating volatiles (atmospheric pollutants migrating inward) and the aroma substances, escaping outward, odor absorption when "empty" CD is used, and controlled release of the active component (antimicrobial, antioxidant, etc.) when the CD complex is applied (*9*).

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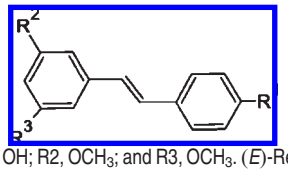
Scheme 1. Structures of Pterostilbene, (*E*)-Resveratrol, and Pinosylvin[a]



[a] Pterostilbene: R1, OH; R2, OCH_3; and R3, OCH_3. (*E*)-Resveratrol: R1, OH; R2, OH; and R3, OH. Pinosylvin: R1, H; R2, OH; and R3, OH.

With regard to this last application, several papers have reported on the complexation of different antimicrobial compounds by CDs (*10*). When a CD−antimicrobial compound molecular complex is exposed to water molecules, its interaction is weakened, and the antimicrobial agent is passively released to the environment. These mechanisms could be used to generate an antimicrobial active packaging and therefore protect fresh-cut produce against bacterial and fungal growth.

In recent years, some papers have been published about the antimicrobial properties of a new type of stilbenoid, pinosylvin, for use in the packaging industry. Pinosylvin (*trans*-3,5-dihydroxystilbene), $C_{14}H_{12}O_2$ (**Scheme 1**), is a naturally occurring stilbene present in the wood pulp of pine and eucalyptus trees and is present in tea oils and herbal remedies (*11*−*14*). Several healthy benefits have been attributed to pinosylvin in recent years. Among its pharmacological properties are a wide range of biological activities, including antimicrobial (*15*), antifungal (*16*), anticancer (*17*), antiinflammatory (*18*), antioxidative (*19*), and antibacterial (*20*) properties.

Despite these beneficial properties, several disadvantages of stilbenoids related to their poor solubility in water, their facility to be oxidized by different agents, or their tendency to be photodegraded have meant that pinosylvin has not been used in active food-packaging films (*21*). For these reasons, the complexation of pinosylvin with different types of molecules, for example, CDs, has been examined to improve its suitability for use in the food-packaging industry. However, the first step is the molecular characterization of the inclusion process of pinosylvin in CDs, as has been realized in this work for first time.

To date, although CDs have been used to complex another type of stilbenoids, the effect of CDs on pinosylvin has not been described in any paper. Indeed, this is the first study in which the complexation between CD and this potent antimicrobial and antifungal is reported. Knowledge of the stoichiometric coefficients and of the complexation constants (K_F) of the CD−pinosylvin complexes is essential if this stilbene is to be used in the food industry.

Bearing the above in mind, the three main objectives of this work were as follows: (i) to analyze the complexation mechanism of pinosylvin with different types of natural (α-, *β*-, and *γ*-CD) and modified (hydroxypropyl-*β*-CD, hydroxyethyl-*β*-CD, methyl-*β*-CD, or hydroxypropyl-*γ*-CD) CDs under various experimental conditions of temperature and pH; (ii) to calculate the stoichiometry, K_F, values and thermodynamic parameters for the CD−pinosylvin complexes; and (iii) to compare the effect of the structure of some compounds of the stilbenoids family, such as (*E*)-resveratrol and pterostilbene, on both the stoichiometry and the K_F values. To perform the study, a method that makes use of changes in fluorescence spectroscopic properties of pinosylvin in the presence of CDs was used.

EXPERIMENTAL PROCEDURES

Materials. Pinosylvin and pterostilbene were purchased from Sequoia Research Products Limited (Pangbourne, U.K.). (*E*)-Resveratrol was from Sigma-Aldrich (Madrid, Spain). Stilbenes are sensitive to light, and irradiation of solutions containing the analyte induces the formation of other molecules, which leads to the formation of a highly fluorescent compound. Moreover, these stilbenes are also sensitive to light because of their (*E*) to (*Z*) diastereoisomerization. Because of this, the samples were stored in darkness. All of the CDs tested, natural (α-, *β*-, and *γ*-CD) and modified [hydroxypropyl-*β*-CD (HP-*β*-CD), hydroxyethyl-*β*-CD (HE-*β*-CD), methyl-*β*-CD, or hydroxypropyl-*γ*-CD (HP-*γ*-CD)], were purchased from Sigma-Aldrich and used as received.

Equipment and Experimental Procedures. *Fluorescence Studies*. The excitation wavelength for pinosylvin and pterostilbene was 330 and that for (*E*)-resveratrol was 334, whereas the emissions were 374 for pinosylvin and pterostilbene and 385 for (*E*)-resveratrol. The relative fluorescence intensity values were recorded at 25 ± 0.2 °C. To avoid inner filter effects, 2 mm quartz cells were used. A Kontron SFM-25 spectrofluorometer (Zurich, Switzerland) equipped with a xenon lamp source and thermostatically controlled cells was used to measure the fluorescence intensity in all of the fluorescence experiments. Both the excitation and the emission bandwidths were set at 2 nm.

Temperature Studies. Four temperatures (15 ± 0.2, 20 ± 0.2, 25 ± 0.2, 30 ± 0.2, and 37 ± 0.2 °C) were used to determine the K_F values. To control the temperature, a Thermomixer Comfort (Eppendorf Ibérica, Madrid, Spain) was used. The thermodynamic parameters, $\Delta H°$, $\Delta S°$, and $\Delta G°$, can be estimated using the thermodynamic relationship equation

$$\ln K_F = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R} \quad (1)$$

where K_F is the complexation constant of the inclusion complex, R is the gas constant, T is the temperature, and $\Delta H°$ and $\Delta S°$ are standard enthalpy and entropy changes of complex formation in the system. For a linear plot of ln K_F versus $1/T$, the slope and intercept are $-\Delta H°/R$ and $\Delta S°/R$, respectively.

Using eq 2, the Gibbs free energy change for the interactions that take place during the inclusion process can be determined as follows:

$$\Delta G° = \Delta H° - T\Delta S° \quad (2)$$

RESULTS AND DISCUSSION

Determination of the Stoichiometry and Complexation Constants of the Inclusion Process of Pinosylvin by CDs. Although the interaction between CDs and other stilbenes has been reported in several papers (*22*−*24*), no work has reported on the possible interaction between pinosylvin and CDs of any type. To study the hypothetical interaction between this healthy stilbenoid and CDs, in this paper, we selected the most widely used natural CD (*β*-CD). To quantify the interaction between pinosylvin and this type of natural CD, the K_F was determined using, as an analysis technique, the steady-state fluorescence, which takes into account the changes in the physicochemical state of this compound with the concentration of CD and following the Benesi−Hildebrand method (*25*).

Two types of stoichiometry have been described for the CD−stilbenoids complexes, 1:1 and 2:1 (*22*−*24*). For this reason, the next step of this investigation was to determine the stoichiometry of the *β*-CD−pinosylvin complex.

Assuming that the composition of the complex was 1:1, the following expression can be written:

$$\text{pinosylvin} + \beta\text{-CD} \rightleftarrows \beta\text{-CD}-\text{pinosylvin}$$

The complexation constant, K_F, is given by

$$K_F = \frac{[\beta\text{-CD}-\text{pinosylvin}]}{[\text{pinosylvin}][\beta\text{-CD}]} \quad (3)$$

where [*β*-CD], [pinosylvin], and [*β*-CD−pinosylvin] are equilibrium concentrations.

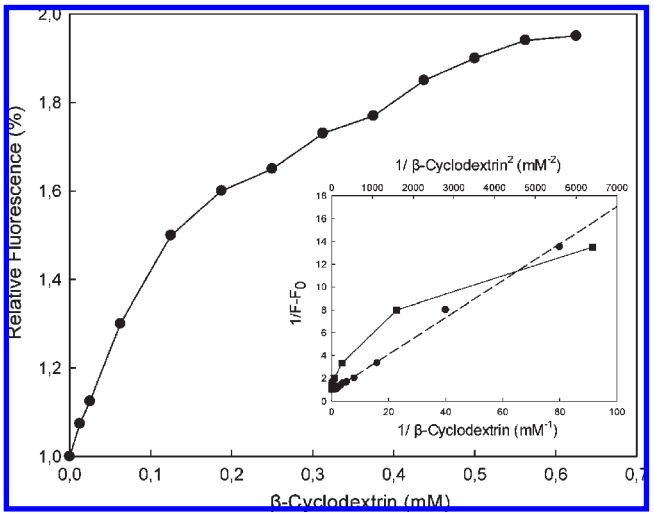


Figure 1. Dependence of emission fluorescence intensities of pinosylvin on β-CD concentrations. [Pinosylvin], 30 μM. (Inset) Double-reciprocal plot of pinosylvin complexed to β-CD for determining the stoichiometry of β-CD−pinosylvin complexes: $1/(F - F_0)$ versus $1/[\beta\text{-CD}]$ (assumption of 1:1 complex) (●); $1/(F - F_0)$ versus $1/[\beta\text{-CD}]^2$ (hypothesis of 1:2 complex) (■).

Using a Benesi−Hildebrand plot, we can calculate the stoichiometry and the K_F value for the 1:1 inclusion complex

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K_F[\beta\text{-CD}]} + \frac{1}{F_\infty - F_0} \quad (4)$$

where F is the observed fluorescence intensity at each β-CD concentration tested, F_0 is the fluorescence intensity of pinosylvin in the absence of β-CD, F_∞ is the fluorescence intensity when all of the pinosylvin molecules are essentially complexed with β-CD, and [β-CD] denotes the β-CD concentration.

A similar expression can be used for the 2:1 inclusion complexes, and a plot of $1/F - F_0$ as a function of $1/[\beta\text{-CD}]^2$ was also analyzed because it was thought that it might provide information about the presence of higher order complexes, especially at higher β-CD concentrations. Assuming the stoichiometry of the inclusion complex to be 2:1, the following expression is obtained (*30*):

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K_{F12}([\beta\text{-CD}])^2} + \frac{1}{F_\infty - F_0} \quad (5)$$

To select which type of inclusion process is carried out between pinosylvin and β-CD, the first step was to study the changes in the pinosylvin emission fluorescence when β-CD was added to the reaction medium. **Figure 1** shows the emission fluorescence of pinosylvin values in the presence of increasing concentrations of β-CD. From these data, the difference in the intensity of the emission fluorescence of pinosylvin in the absence and presence of different amounts of β-CD was calculated. To determine the stoichiometry and the K_F of β-CD−pinosylvin complexes, a representation of the variation in fluorescence intensity at the wavelength band used as a function of β-CD concentration was analyzed according to the Benesi−Hildebrand method (**Figure 1**, inset).

In our study, a plot of $1/F - F_0$ versus $1/[\beta\text{-CD}]$ gave a straight line with a linear correlation higher than 0.99, indicating that the presumed stoichiometry of the β-CD−pterostilbene complexes formed was 1:1 (**Figure 1**, inset, ●). On the other hand, when $1/F - F_0$ was plotted against $1/([\beta\text{-CD}])^2$, a nonlinear relationship was obtained (linear correlation of 0.82) (**Figure 1**, inset, ■),

Table 1. K_F Values and Correlation Coefficients Arising from Equations 4 and 5 (for 1:1 and 1:2 Pinosylvin−Natural CD Complexes, Respectively) at 25 ± 0.2 °C at pH 7.0

			correlation coefficient	
complex	K_F (M^{-1})	inner diameter (Å)	1:1 using eq 4	1:2 using eq 5
pinosylvin−α-CD	4024 ± 135	4.7−5.2	0.99	0.89
pinosylvin−β-CD	5181 ± 233	6.0−6.4	0.99	0.82
pinosylvin−γ-CD	3084 ± 119	7.5−8.3	0.99	0.84

which indicates that the stoichiometry of the inclusion complex is not 2:1. This type of stoichiometry 1:1 has been reported for other CD−stilbenoid complexes such as (*E*)-resveratrol (*22*, *23*) or pterostilbene (*24*) but differs from the stoichiometry 2:1 published for *trans*-stilbene (*24*).

To quantify for the first time the interaction between pinosylvin and CD, the experimental data were fitted to eq 4, whereas the K_F value for pH 7.0 was calculated as 5181 ± 233 M^{-1}. These results are in good agreement with those previously obtained for the 1:1 complexes between β-CD and several compounds with structures similar to that of pinosylvin (*22*−*24*).

Study of the Complexation of Pinosylvin by Natural CDs: Effect of the CD Structure on Complexation Constants. To characterize the interaction between the pinosylvin and the host CD at a molecular level, the next step of our investigation was to determine the K_F values between pinosylvin and several types of CD with different structures, sizes, and glucose unit numbers. Three types of natural CD with GRAS status and approved recently as additives in the European Union (α-, β-, and γ-CD) were used to this end. Indeed, the three natural CDs have been included in the European lists of additives approved for food use, and the corresponding E-numbers assigned are E-457, E-459, and E-458, respectively. As in the previous section, when determining the K_F values between β-CD and pinosylvin, we fitted the values of relative intensity calculated experimentally to the previously equations mentioned. The K_F values for different natural species are shown in **Table 1**. It can be observed that the highest K_F value belonged to β-CD, followed by α-CD and, finally, γ-CD.

At the molecular level, our data show that the inner diameter of the CD formed by six units of glucose (β-CD, 6.0−6.4 Å) fitted pinosylvin better than the inner diameter of five units (α-CD, 4.7−5.2 Å) or seven units (γ-CD, 7.5−8.3 Å) of glucose. The fact that β-CD was the optimum natural CD for complexing pinosylvin is in good agreement with most of the papers, which compare the complexation of several stilbenoid compounds with CDs (*22*, *23*). Because β-CD was the most effective CD for complexing pinosylvin, this natural CD was chosen to continue the investigation.

Determination of the K_F Values for the Complexation of Pinosylvin by Modified CDs. The effect of modifying β-CD by adding different functional groups to the macrocyclic ring on the K_F values was evaluated. Our results showed significant differences when HP-β-CD, HE-β-CD, methyl-β-CD, or HP-γ-CD was used instead of β-CD. In all cases, the natural CDs showed lower K_F values than the modified CDs tested. As expected, the relative fluorescence values of pinosylvin increased with the concentration of all four modified CDs (**Figure 2**). However, the addition of increasing concentrations of modified CDs led to greater increases in the relative intensity fluorescence values than when increasing concentrations of β-CD were used. Our data showed that HP-β-CD presented the highest K_F value of all of the modified CDs tested, followed by HE-β-CD, methyl-β-CD, and, finally, HP-γ-CD (**Figure 2**, inset). Several factors may influence the K_F values for the pinosylvin-modified CDs

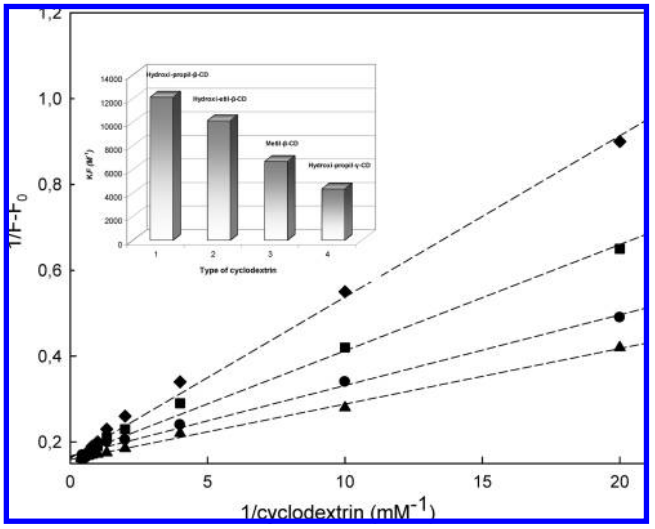


Figure 2. Double-reciprocal plot of pinosylvin complexed to modified CDs HP-β-CD (▲), HE-β-CD (●), methyl-β-CD (■), and HP-γ-CD (◆). (Inset) Effect of the structure of modified and natural CDs on the complexation constant (K_F) values of pinosylvin−CD complexes at 25 ± 0.2 °C in 0.1 M sodium phosphate buffer, pH 7.0. [Pinosylvin], 30 μM.

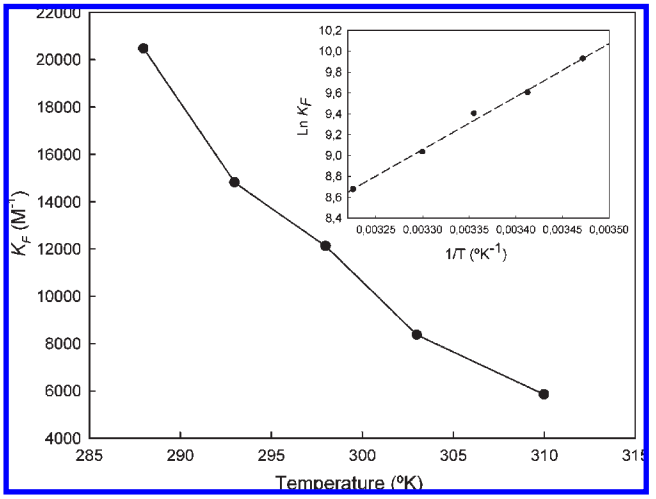


Figure 3. Effect of temperature on the complexation constant (K_F) values of pinosylvin−HP-β-CD complexes at pH 7.0. [Pinosylvin], 30 μM. (Inset) Van't Hoff plot (ln K_F versus 1/T) for pinosylvin−HP-β-CD complexes in 0.1 M sodium phosphate buffer, pH 7.0.

interactions. In this investigation, the length of the aliphatic chain of the β-CD substituent influenced the strength of the complexation. Thus, for a natural CD, the greater the number of carbon atoms in the substituent, the higher the K_F value for the resulting complex.

The hydrophobicity of the β-CD channel increases with the modification because it occurs principally at position 2 of the sugar residues situated on one side of the torus at the edge and orientated inward. Indeed, the higher K_F observed for the modified CDs−pinosylvin complexes could be due to the hydrophobic interactions with one side of the CD molecule (that bearing the methyl, ethyl, or hydroxypropyl groups). The behavior of K_F may also be explained by the substantial changes resulting from the substitution of the internal −OH groups in the hydrophobicity of the CD torus.

The fact that HP-β-CD was the best type of CD to complex pinosylvin coincides with the results shown by other authors concerning the use of HP-β-CD to complex other stilbene compounds (*26*). For this reason, we selected HP-β-CD to study the complexation of pinosylvin by CDs.

Effect of Temperature on the Complexation of Pinosylvin by HP-β-CD. The effect of temperature on the complexation process is one of the main factors to be taken into account when a host−guest complex is used in the food or pharmaceutical industry. Therefore, the next step in this work was to study the influence of the temperature on the K_F values of the HP-β-CD−pinosylvin complexes.

Different papers have reported that temperature can increase or decrease the strength of inclusion of a guest molecule. Generally, an increase in the medium temperature produces a dissociation of the inclusion complexes, and the K_F values decrease (*27*). However, the inclusion of another group of molecules such as polyunsaturated fatty acids in the cavity of CDs is favored by high temperatures (*28*). For this reason, the effect of temperature on the CD−pinosylvin complexes was clarified by studying the K_F values for the HP-β-CD−pinosylvin complexes at four different temperatures: 15 ± 0.2, 25 ± 0.2, 30 ± 0.2, and 37 ± 0.2 °C. The results can be observed in the **Figure 3**; the K_F values obtained at 15 ± 0.2, 25 ± 0.2, 30 ± 0.2, and 37 ± 0.2 °C were 20460 ± 1036, 14800 ± 965, 12112 ± 761, 8364 ± 569, and 5839 ± 472 M^{-1}, respectively.

This strong decrease in the K_F values of the HP-β-CD−pinosylvin complexes when the temperature is increased might be interpreted as a lower degree of interaction at higher temperatures, possibly due to the fact that hydrogen bonds are usually weakened by heating.

Thermodynamic Study of the Complexation of Pinosylvin by HP-β-CD. To obtain information on mechanistic aspects of pinosylvin's affinity for HP-β-CD, a van't Hoff plot (eq 1) was used to calculate the main thermodynamic parameters of the complexation process ($\Delta H°$, $\Delta S°$, and $\Delta G°$ at 25 ± 0.2 °C). To reach this objective, the Ln K_F was plotted versus $1/T$, and a lineal representation was obtained with a correlation coefficient higher than 0.99 (**Figure 3**, inset).

These results led to three main conclusions concerning the nature of the complexation of pinosylvin by HP-β-CD: (i) The process is exothermic. he negative values obtained for enthalpy changes (−42 ± 1 kJ mol^{-1}) indicate the exothermic nature of the interaction processes of pinosylvin with HP-β-CD. This behavior is typical of hydrophobic interactions, van der Waals interactions, the displacement of water molecules from the cavity of HP-β-CD, or the formation of hydrogen bonds. (ii) The process presents a negative value for entropy changes (−64 ± 2 J mol^{-1} K^{-1}) due to a decrease in the translational and rotational degrees of freedom of the complexed pinosylvin as compared with the free ones. (iii) The process is spontaneous. The negative value obtained for the Gibbs free energy change (−23 ± 1 kJ mol^{-1}) for the interactions that take place during the inclusion process at 25 ± 0.2 °C indicates that the inclusion process is spontaneous.

Effect of pH on the Complexation of Pterostilbene by HP-β-CD. The behavior of the HP-β-CD−pinosylvin complexes at different protonation states of the guest molecule must be taken into account when this CD complex is used in the food or pharmaceutical industry. For this reason, in this section, we study the effect of the medium pH on the complexation of pinosylvin by HP-β-CD, calculating the K_F values for this type of complex in the pH range of 5.5−10.0. When the medium pH was between 5.5 and 8.5, the K_F values remained stable, with a value around 12100 ± 760 M^{-1} (**Figure 4**). However, when the medium pH was increased from 8.5 to 11.5, the K_F values decreased to about 5000 ± 256 M^{-1}, as happens during the titration of a weak ionizable group. Because hydrogen bonding is one of the most important types of interactions in the stabilization of inclusion complexes,

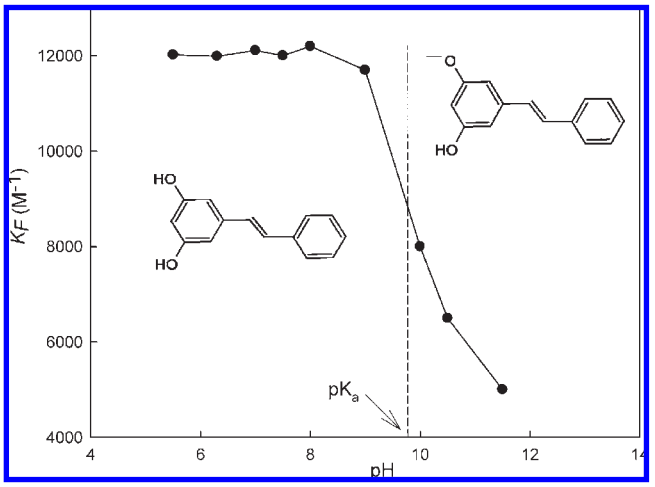


Figure 4. Effect of pH on the complexation constant (K_F) values of pinosylvin−HP-β-CD complexes at 25 ± 0.2 °C. [Pinosylvin], 30 μM.

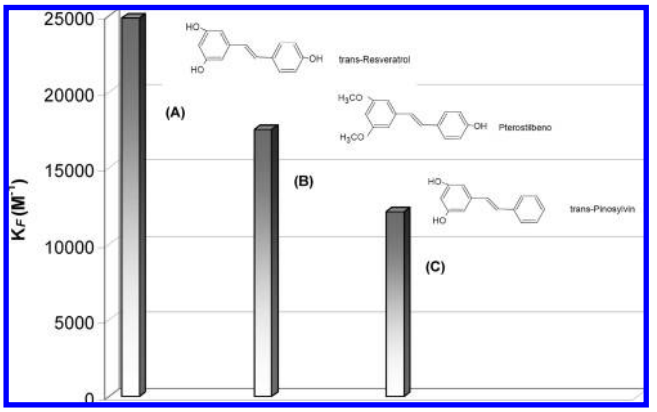


Figure 5. Effect of the structure of (*E*)-resveratrol (A), pterostilbene (B), and pinosylvin (C) on the complexation constant (K_F) values of stilbenoid−HP-β-CD complexes at 25 ± 0.2 °C in 0.1 M sodium phosphate buffer, pH 7.0. [Stilbenoids], 30 μM.

this strong decay in the complexation constant at high pH values may be attributed to the hypothetical formation of a hydrogen bond between one of the hydroxyl groups of the pinosylvin and the hydrophilic groups of CD at pH values below the pK_a value. As can be observed in **Figure 4**, a significant decrease in the K_F value occurs in the pH region where the stilbenoids begin deprotonation of their hydroxyl groups.

Recently, López-Nicolás and García-Carmona (*29*) reported three pK_a values for (*E*)-resveratrol (pK_{a1}, 8.8; pK_{a2}, 9.8; and pK_{a3}, 11.4). The first pK_a is associated with the deprotonation of 4-OH because the abstraction of 4-H is easier than that of 3-H and 5-H. The second pK_a indicates the deprotonation of 3-OH or 5-OH (the 3- and 5-positions have the same structures because the molecule is symmetric). The third pK_a indicates the deprotonation of 5-OH or 3-OH. Our results showed that the decay in the K_F value of pinosylvin at pH 8.5−11.5 may indicate the existence of a pK_a of 9.8 for this stilbenoid, which coincides with that presented by López-Nicolás and García-Carmona (*29*) for the pK_{a2} of (*E*)-resveratrol.

The fact that the K_F values were higher at pH values lower than the pK_a value of pinosylvin shows that the complexes between HP-β-CD and the protonated form of pinosylvin were more stable than the interaction with the deprotonated forms of this antifungal and antimicrobial compound. These results are of great interest for both the food and pharmaceutical industries because several papers have reported that the protonated structures of stilbenoids have important beneficial biological effects for human health (*30*).

Effect of the Structure of Different Derivatives of Stilbene on Its Complexation by HP-β-CD. Recently, Perecko et al. (*31*) studied the structure−efficiency relationship in derivatives of stilbene, comparing (*E*)-resveratrol, pinosylvin, and pterostilbene. They concluded that the presence of different functional groups in the molecules of stilbenoids influences their antioxidative effect. Moreover, the modification of these functional groups may result in derivatives with the required antioxidative properties, targeting mainly extracellular reactive oxygen species, which are responsible for tissue damage during chronic inflammation. For this reason and because of the importance of the structure of these compounds in their potential health benefits, the influence of the structure of these three stilbene derivatives on the K_F values needs to be studied. As cited previously, in recent years, the complexation of different stilbenoids by several types of natural and modified CDs has been reported. However, the different methods used to study the inclusion process have resulted in strong differences in the observed K_F values. In our study, both the K_F values and the stoichiometry for the complexation of three derivatives of stilbene [pinosylvin, (*E*)-resveratrol, and pterostilbene], which differ in the number of the hydroxyl groups and in the type of substituents of the aromatics ring, were determined (**Scheme 1**).

Figure 5 shows the relative K_F values for the complexes between HP-β-CD and the three derivatives of stilbene studied in this section. The complexes formed between all of the stilbenoids tested with HP-β-CD presented a 1:1 stoichiometry. Moreover, a comparison of the K_F values (**Figure 5**) showed that the interaction was more effective for the HP-β-CD−(*E*)-resveratrol complexes, followed by the HP-β-CD−pterostilbene complexes, and, finally, the lowest K_F value was found for the HP-β-CD−pinosylvin complexes. Among the causes of this behavior may be the hydrophobicity, resonance structure of the guest molecules, or the type of substituent of the aromatic rings. Indeed, (*E*)-resveratrol, the stilbenoid that presents the highest K_F value, shows a resonance structure that produces a high stability in this type of stilbene, which is not shown by pterostilbene or pinosylvin, and may improve by its complexation by CDs. Concerning the K_F values determined for the complexes between HP-β-CD and pterostilbene or pinosylvin, the higher hydrophobicity of pterostilbene produced by the presence of two methyl substituents leads to a better fit of the guest molecule in the HP-β-CD cavity.

The use of antimicrobial, antifungal, and antioxidant agents as components of active and intelligent packaging is one of the main objectives of the food industry. However, problems concerning the physicochemical properties of some of these components have meant that some potent antimicrobial, antifungal, and antioxidant agents, such as pinosylvin, have not been used in active food-packaging films. Indeed, pinosylvin shows very poor solubility in water, and more importantly, it is easily oxidized by several prooxidant agents. For these reasons, complexation of pinosylvin with types of molecules, such as CDs, that can reduce the release of impurities and undesired volatile products, improve the barrier function of the packaging material, and protect the antimicrobial, antifungal, and antioxidant agent from degradation is desirable. Toward this aim, we have made a molecular characterization of the inclusion process of pinosylvin in CDs. Although the stoichiometry of the complex is 1:1 for all of the conditions used, our results show that the K_F values for the stilbenoid−CD complexes are strongly dependent on several factors, such as temperature, pH, type of CD, and structure of

the guest molecule. Besides the above-mentioned applications in the food-packaging industry, another potential application for the resulting pinosylvin−CD complexes may be in the food and pharmaceutical ingredient industry as a nutraceutical due to their high solubility and stability and because the pinosylvin−CD complexes may slow the rapid metabolism and elimination of pinosylvin, improving its bioavailability, as has been demonstrated for other stilbenoid complexes.

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